

-continued

Asn	Ser	Phe	Gly	Asp	His	Arg	Ile	Ala	Met	Ser	Leu	Ala	Val	Ala	Gly
385					390					395					400
Val	Arg	Ala	Ala	Gly	Glu	Leu	Leu	Ile	Asp	Asp	Gly	Ala	Val	Ala	Ala
			405						410					415	
Val	Ser	Met	Pro	Gln	Phe	Arg	Asp	Phe	Ala	Ala	Ala	Ile	Gly	Met	Asn
			420					425					430		
Val	Gly	Glu	Lys	Asp	Ala	Lys	Asn	Cys	His	Asp					
		435					440								

We claim:

1. An isolated DNA molecule which encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

2. A DNA molecule of claim 1 having the sequence of SEQ ID NO:2.

3. A DNA molecule of claim 1 having the sequence of SEQ ID NO:9.

4. A recombinant, double-stranded DNA molecule comprising in sequence:

a) a promoter which functions in plant cells to cause the production of an RNA sequence;

b) a structural DNA sequence that causes the production of an RNA sequence which encodes a EPSPS enzyme having the sequence domains:

-R-X₁-H-X₂-E (SEQ ID NO:37), in which

X₁ is G, S, T, C, Y, N, Q, D or E;

X₂ is S or T; and

-G-D-K-X₃ (SEQ ID NO:38), in which

X₃ is S or T; and

-S-A-Q-X₄-K (SEQ ID NO:39), in which

X₄ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

-N-X₅-T-R (SEQ ID NO:40), in which

X₅ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

c) a 3' non-translated region which functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence;

where the promoter is heterologous with respect to the structural DNA sequence and adapted to cause sufficient expression of the encoded EPSPS enzyme to enhance the glyphosate tolerance of a plant cell transformed with the DNA molecule.

5. A DNA molecule of claim 4 in which the structural DNA sequence encodes a fusion polypeptide comprising an amino-terminal chloroplast transit peptide and the EPSPS enzyme.

6. A DNA molecule of claim 4 in which X₁ is D or N; X₂ is S or T; X₃ is S or T; X₄ is V, I or L; and X₅ is P or Q.

7. A DNA molecule of claim 6 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

8. A DNA molecule of claim 5 in which X₁ is D or N; X₂ is S or T; X₃ is S or T; X₄ is V, I or L; and X₅ is P or Q.

9. A DNA molecule of claim 8 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

10. A DNA molecule of claim 8 in which the EPSPS sequence is SEQ ID NO:3.

11. A DNA molecule of claim 10 in which the promoter is a plant DNA virus promoter.

12. A DNA molecule of claim 11 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.

13. A DNA molecule of claim 10 in which the structural DNA sequence encodes a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:15.

14. A DNA molecule of claim 13 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

15. A method of producing genetically transformed plants which are tolerant toward glyphosate herbicide, comprising the steps of:

a) inserting into the genome of a plant cell a recombinant, double-stranded DNA molecule comprising:

i) a promoter which functions in plant cells to cause the production of an RNA sequence,

ii) a structural DNA sequence that causes the production of an RNA sequence which encodes an EPSPS enzyme having the sequence domains:

-R-X₁-H-X₂-E (SEQ ID NO:37), in which

X₁ is G, S, T, C, Y, N, Q, D or E;

X₂ is S or T; and

-G-D-K-X₃ (SEQ ID NO:38), in which

X₃ is S or T; and

-S-A-Q-X₄-K (SEQ ID NO:39), in which

X₄ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

-N-X₅-T-R (SEQ ID NO:40), in which

X₅ is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

iii) a 3' non-translated DNA sequence which functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence;

where the promoter is heterologous with respect to the structural DNA sequence and adapted to cause sufficient expression of the polypeptide to enhance the glyphosate tolerance of a plant cell transformed with the DNA molecule;

b) obtaining a transformed plant cell; and

c) regenerating from the transformed plant cell a genetically transformed plant which has increased tolerance to glyphosate herbicide.

16. A method of claim 15 in which X₁ is D or N; X₂ is S or T; X₃ is S or T; X₄ is V, I or L; and X₅ is P or Q.

17. A method of claim 16 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

18. A method of claim 15 in which the structural DNA sequence encodes a fusion polypeptide comprising an amino-terminal chloroplast transit peptide and the EPSPS enzyme.

19. A method of claim 18 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.

20. A method of claim 19 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:42 and SEQ ID NO:44.

21. A method of claim 19 in which the EPSPS enzyme is that set forth in SEQ ID NO:3.

22. A method of claim 21 in which the promoter is from a plant DNA virus.

23. A method of claim 22 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.

24. A glyphosate-tolerant plant cell comprising a DNA molecule of claims 5, 8 or 10.

25. A glyphosate-tolerant plant cell of claim 24 in which the promoter is a plant DNA virus promoter.

26. A glyphosate-tolerant plant cell of claim 25 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.

27. A glyphosate-tolerant plant cell of claim 24 selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

28. A glyphosate-tolerant plant comprising plant cells of claim 27.

29. A glyphosate-tolerant plant of claim 28 in which the promoter is from a DNA plant virus promoter.

30. A glyphosate-tolerant plant of claim 29 in which the promoter is selected from the group consisting of CaMV35S and FMV35S promoters.

31. A glyphosate-tolerant plant of claim 30 selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

32. A method for selectively controlling weeds in a field containing a crop having planted crop seeds or plants comprising the steps of:

a) planting the crop seeds or plants which are glyphosate-tolerant as a result of a recombinant double-stranded DNA molecule being inserted into the crop seed or plant, the DNA molecule having:

i) a promoter which functions in plant cells to cause the production of an RNA sequence,

ii) a structural DNA sequence that causes the production of an RNA sequence which encodes an EPSPS enzyme having the sequence domains:

-R- X_1 -H- X_2 -E-(SEQ ID NO:37), in which

X_1 is G, S, T, C, Y, N, Q, D or E;

X_2 is S or T; and

-G-D-K- X_3 -(SEQ ID NO:38), in which

X_3 is S or T; and

-S-A-Q- X_4 -K-(SEQ ID NO:39), in which

X_4 is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

-N- X_5 -T-R-(SEQ ID NO:40), in which

X_5 is A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y or V; and

iii) a 3' non-translated DNA sequence which functions in plant cells to cause the addition of a stretch of polyadenyl nucleotides to the 3' end of the RNA sequence

where the promoter is heterologous with respect to the structural DNA sequence and adapted to cause sufficient expression of the EPSPS enzyme to enhance the glyphosate tolerance of the crop plant transformed with the DNA molecule; and

b) applying to the crop and weeds in the field a sufficient amount of glyphosate herbicide to control the weeds without significantly affecting the crop.

33. A method of claim 32 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.

34. A method of claim 33 in which the structural DNA sequence encodes an EPSPS enzyme selected from the sequences as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:42 and SEQ ID NO:44.

35. A method of claim 32 in which the structural DNA sequence encodes a fusion polypeptide comprising an amino-terminal chloroplast transit peptide and the EPSPS enzyme.

36. A method of claim 35 in which X_1 is D or N; X_2 is S or T; X_3 is S or T; X_4 is V, I or L; and X_5 is P or Q.

37. A method of claim 36 in which the structural DNA sequence encodes an EPSPS enzyme selected from the sequences as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

38. A method of claim 36 in which the DNA molecule encodes an EPSPS enzyme as set forth in SEQ ID NO:3.

39. A method of claim 38 in which the DNA molecule further comprises a promoter selected from the group consisting of the CaMV35S and FMV35S promoters.

40. A method of claim 39 in which the crop plant is selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

41. A DNA molecule of claim 5 in which the structural DNA sequence encodes a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 and SEQ ID NO:17.

42. A DNA molecule of claim 41 in which the chloroplast transit peptide is encoded by a DNA sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16.

43. A DNA molecule of claim 5 in which the structural DNA sequence encodes a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:15.

44. A DNA molecule of claim 43 in which the chloroplast transit peptide is encoded by a DNA sequence selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:14.

45. A DNA molecule of claim 41 in which the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters.

46. A DNA molecule of claim 42 in which the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters.

47. A DNA molecule of claim 43 in which the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters.

48. A DNA molecule of claim 44 in which the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters.

49. A DNA molecule of claim 45 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

50. A DNA molecule of claim 46 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

51. A DNA molecule of claim 47 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

52. A DNA molecule of claim 48 in which the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

53. A DNA molecule of claim 49 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44.

54. A DNA molecule of claim 50 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44.

55. A DNA molecule of claim 51 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44.

56. A DNA molecule of claim 52 in which the structural DNA sequence encodes an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44.

57. A DNA molecule of claim 53 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

58. A DNA molecule of claim 54 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

59. A DNA molecule of claim 55 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

60. A DNA molecule of claim 56 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

61. A DNA molecule of claim 49 in which the structural DNA sequence encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

62. A DNA molecule of claim 50 in which the structural DNA sequence encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

63. A DNA molecule of claim 51 in which the structural DNA sequence encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

64. A DNA molecule of claim 52 in which the structural DNA sequence encodes an EPSPS enzyme having the sequence of SEQ ID NO:3.

65. A DNA molecule of claim 61 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

66. A DNA molecule of claim 62 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

67. A DNA molecule of claim 63 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

68. A DNA molecule of claim 64 in which the structural DNA sequence contains an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

69. A glyphosate-tolerant plant cell of claim 25 in which:

(a) the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters;

(b) the structural DNA sequence encodes:

(i) a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 and SEQ ID NO:17; and

(ii) an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44; and

(c) the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

70. A glyphosate-tolerant plant cell of claim 69 in which the structural DNA sequence comprises:

(a) a chloroplast transit peptide encoding DNA sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16; and

(b) an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

71. A glyphosate-tolerant plant cell of claim 69 in which the structural DNA sequence comprises:

(a) a chloroplast transit peptide encoding DNA sequence selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:14; and

(b) a DNA sequence encoding an EPSPS enzyme having the sequence of SEQ ID NO:3.

72. A glyphosate-tolerant plant cell of claim 71 in which the structural DNA sequence comprises an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

73. A glyphosate-tolerant plant cell of claim 71 selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eukalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

74. A glyphosate-tolerant plant comprising a DNA molecule of claims 5, 8 or 10 in which:

(a) the promoter is selected from the group consisting of CaMV 35S and FMV 35S promoters;

(b) the structural DNA sequence encodes:

(i) a chloroplast transit peptide selected from the group consisting of SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 and SEQ ID NO:17; and

(ii) an EPSPS enzyme selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:42 and SEQ ID NO:44; and

(c) the 3' non-translated region is selected from the group consisting of the NOS 3' and the E9 3' non-translated regions.

75. A glyphosate-tolerant plant of claim 74 in which the structural DNA sequence comprises:

(a) a chloroplast transit peptide encoding DNA sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16; and

(b) an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:41 and SEQ ID NO:43.

76. A glyphosate-tolerant plant of claim 75 in which the structural DNA sequence comprises:

(a) a chloroplast transit peptide encoding DNA sequence selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:14; and

(b) a DNA sequence encoding an EPSPS enzyme having the sequence of SEQ ID NO:3.

77. A glyphosate-tolerant plant of claim 76 in which the structural DNA sequence comprises an EPSPS encoding sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:9.

78. A glyphosate-tolerant plant of claim 77 selected from the group consisting of corn, wheat, rice, barley, soybean, cotton, sugarbeet, oilseed rape, canola, flax, sunflower, potato, tobacco, tomato, alfalfa, poplar, pine, eucalyptus, apple, lettuce, peas, lentils, grape and turf grasses.

79. A seed of a glyphosate-tolerant plant of claim 28.

80. A seed of a glyphosate-tolerant plant of claim 31.

81. A seed of a glyphosate-tolerant plant of claim 75.

82. A seed of a glyphosate-tolerant plant of claim 76.

83. A seed of a glyphosate-tolerant plant of claim 77.

84. A seed of a glyphosate-tolerant plant of claim 78.

85. A seed of a glyphosate-tolerant plant of claim 79.

86. A transgenic soybean plant which contains a heterologous gene which encodes an EPSPS enzyme having a K_m for phosphoenolpyruvate (PEP) between 1 and 150 μ M and a $K_i(\text{glyphosate})/K_m(\text{PEP})$ ratio between about 2 and 500, said plant exhibiting tolerance to N-phosphonomethylglycine herbicide at a rate of 1 lb/acre without significant yield reduction due to herbicide application.

87. Seed of a soybean plant of claim 86.

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